Small heat shock proteins genes are differentially expressed in distinct varieties of common bean

Jean Luiz Simões-Araújo[1,2]*, Norma Gouvêa Rumjanek[1] and Márcia Margis-Pinheiro[2]

*[1]Embrapa-Agrobiologia - BR 465, Km 47 (Antiga Estrada Rio São Paulo), CP 74505, CEP 23851-970, Seropédica, RJ, Brasil; [2]Laboratório de Genética Molecular Vegetal - Departamento de Genética Universidade Federal do Rio de Janeiro CP 68011, CEP 21941-970, Ilha do Fundão, Rio de Janeiro, RJ, Brasil; * Corresponding author: jean@cnpab.embrapa.br*
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Plants respond to temperature stress by synthesizing a set of heat shock proteins (HSPs), which may be responsible for the acquisition of thermotolerance. In this study, the induction of small HSPs (sHSPs) in eight common bean varieties was evaluated by Northern blot analysis using the W HSP 16.9 cDNA as heterologous probe. Cowpea was used, as a positive control since this plant, as opposed to common bean, is known to grow well under high temperature regimes such as that found in the Brazilian semi-arid region. After the growth period, the plants were submitted to two h of heat shock at 40 ºC. All varieties tested were able to induce sHSP mRNAs that hybridized with W HSP 16.9 probe. However, significant kinetic differences were found when comparing different varieties. SHSP mRNA levels induced after heat shock in cowpea was higher than the levels observed on the bean varieties displaying the highest expression of these proteins. Besides, the sHSP expression was also assessed at the protein accumulation level by Western-blot analysis for cowpea and both IPA 7 and Negro Argel varieties of bean plants. The revealed protein pattern confirmed that sHSPs are differentially expressed in distinct varieties of common bean according their heat stress tolerance.

Key words: common bean, heat shock proteins, HSPs, thermotolerance, temperature stress.

Expressão diferenciada de proteínas de choque térmico de baixo peso molecular em distintos cultivares de feijoeiro: As plantas respondem ao estresse provocado por temperaturas elevadas através da síntese de um grupo de proteínas denominadas proteínas de choque térmico (Heat Shock Proteins-HSPs) que estão associadas com a obtenção de termotolerância. Nesse estudo, foi avaliada a indução de proteínas de choque térmico de baixo peso molecular (sHSPs) em oito cultivares de feijoeiro através de "Northern blot" utilizando o cDNA da W HSP 16,9 como uma sonda heteróloga. Caupi foi utilizado como controle, uma vez que, ao contrário do feijoeiro, é uma espécie que se apresenta bem adaptada às condições de temperaturas elevadas como as observadas no Semi-árido brasileiro. Após um período de crescimento, as plantas foram submetidas a um período de duas horas de choque térmico a 40 °C. Todas as variedades avaliadas foram capazes de induzir mRNAs para sHSP que hibridizou com a sonda W HSP 16,9. Entretanto, foi observada uma diferença significativa no padrão de indução entre as diferentes variedades. Os níveis de mRNA e sHSPs induzidos em caupi após o choque térmico foram bem maiores que os observados para as variedades de feijoeiro. Além disso, a expressão de sHSPs foi também avaliada em relação ao acúmulo de proteínas através da análise de "Western-blot" para caupi e as variedades de feijoeiro IPA 7 e Negro Argel. O padrão de proteínas observado confirma que as sHSPs são diferencialmente expressas em diferentes variedades de feijoeiro de acordo com a tolerância ao estresse térmico.

Palavras-chave: estresse térmico, feijão, HSPs, proteínas de choque térmico, termotolerância.

INTRODUCTION

Cells of several organisms induce a set of proteins, the heat shock proteins (HSPs), when exposed not just to high temperature but also to other stresses such as heavy metal contamination, water deficit and presence of pathogens among others (Vierling, 1991). Plants are

generally submitted to different kinds of stresses because it is very uncommon that optimal environmental conditions are attended (Howarth and Ougham, 1993). In tropical areas where excesses of radiation are common, high air and soil temperatures tends to be the most important factor reducing plant growth and yield. Furthermore, under field conditions, high temperature stress is generally associated to reduced water supply, which limits plant productivity even more.

Induction of HSPs seems to be a universal response to temperature stress being observed on all organisms ranging from bacteria to human beings (Vierling, 1991; Parsell and Lindquist, 1993). A comparison of the response to heat stress in different organism has shown that it is highly conserved and the molecular mechanisms of HSP gene induction have many similarities among diverse eukaryotes. Furthermore, the major HSPs are highly homologous among distinct organisms (Vierling, 1991).

HSPs are generally designated by their approximate molecular weights in kDa as HSP110, HSP90, HSP70, HSP60 and Low Molecular Weight HSPs (15–30 kDa), designated small heat shock proteins (sHSP) (Vierling, 1991; Waters et al., 1996; Sun et al., 2002). Although plants synthesize a similar set of high molecular weight HSPs, most of the translation capacity is devoted to the synthesis of the sHSPs (Mansfield, 1987). As far as it is known, all plant sHSPs are encoded by six nuclear gene families, each gene family corresponding to proteins found in distinct cellular compartments: cytosol (class I and class II), chloroplast, endoplasmic reticulum, mitochondria and membranes (Waters et al., 1996). Higher plants have at least 20 sHSPs and same species may have up to 40 different sHSPs (Vierling, 1991). Under heat stress conditions, the level of expression of the class I sHSPs in soybean can reach more than 1 % of the total cellular protein (Hsieh et al., 1992). The wide diversification and abundance of sHSPs in plants may reflect adaptation to temperature stress (Water, 1996).

Some sHSPs are also expressed in some cells under cyclic or developmental control (Hopf, 1992). In the absence of environmental stress, the sHSP expression in plants is restricted to certain stages of development such as embryogenesis, germination, pollen development, and fruit maturation (Sun et al., 2002). Although the total physiological functions of HSPs have not been completely understood, there is considerable evidence to show that the acquisition of thermotolerance is correlated with synthesis and accumulation of HSPs (Lin et al., 1984; Weng and Nguyen, 1992; Jinn et al., 1993; Schirmer et al., 1994; Park et al., 1996; Prändl et al., 1998; Ristic et al., 1998; Joe et al., 2000). There is, however, considerable variation in the patterns of HSP production among different species, and even among individuals of the same specie (Ristic et al., 1991; Wood et al., 1998).

The acquisition of thermotolerance appears to depend not only upon the synthesis of HSPs but also on their cellular localization (Lin et al., 1984; Chou et al., 1989; Heckathorm et al., 1999; Korotaeva et al., 2001). Three observations strengthen this hypothesis: (a) the induction of HSPs has been characterized as an extremely fast and intense response, (b) the induction of HSPs indicates that the organism is being submitted to stress condition and, (c) the synthesis of HSPs is correlated to the induction of tolerance to temperature in a wide variety of cells and organisms (Parsell and Lindquist, 1993).

Common bean (*Phaseolus vulgaris*) is an important food crop in tropical countries. However, common bean is especially sensitive to high temperatures and even short periods under high temperature may reduce plant growth (Lin and Markhart III, 1996), limit nodulation and dinitrogen fixation (Piha and Munns, 1987; Hernandez-Armenta, 1989) causing significant reduction of productivity. The synthesis of some HSPs was already detected in common bean. Vidal et al. (1993) isolated and characterized a HSP 70 kDa related to mitochondria membrane, which plays a role as a molecular chaperone. Chrispeels and Greenwood (1987) using protein labeled with L-[^{3}H]-leucin found a 90 kDa and three sHSPs in common bean cotyledons submitted to 43 ºC. Süss and Yordanov (1986) suggested that the interaction of HSP 22 kDa with the chloroplast external membrane can influence the composition of the membrane and decrease its fluidity, probably increasing the efficiency of the ATP transport. In this case, expression of sHSPs would be fundamental for tolerance to high temperatures.

The study of induced changes by high temperature on gene activation, expression, transcription and translation may help to understand the ability of plants to tolerate environmental heat stress that often occur during the acclimation process (Howarth and Ougham, 1993; Bray, 1997). In this study, we have used an heterologous probe for Northern hybridization to determine the induction and

accumulation of HSP mRNAs in different bean varieties submitted to heat stress. The goal was evaluate the tolerance of different common bean varieties to high temperature stress by analyzing their capacity of induction of bean sHSP expression.

MATERIAL AND METHODS

Plant material and growth conditions: Eight common bean (*Phaseolus vulgaris* L.) varieties were selected for this study according to previous experiments performed at Embrapa Agrobiologia (unpublished date) and to the work developed by Singh (1989), which classified bean varieties according to the cultivation region, growth habit, size and seed colors and type of the phaseolin protein (table 1). The following common bean varieties were used: IPA 7, Negro Argel, Jamapa, Chimaltenango negro, Flor de Mayo, Cacahuate, Cranberry and Canario. As cowpea (*Vigna unguiculata*) is known by its tolerance to several environmental stresses including high temperature, it was selected as an indicator of tolerance.

Common bean and cowpea (*V. unguiculata* cv. IPA 206) seeds were surface-sterilized with $HgCl_2$ (1:500) and then germinated on sterile water-agar (15 $g.L^{-1}$) for 3 d (Vincent, 1970). Soon after germination, each seedling was transferred to a plastic recipient (200 mL), containing vermiculite and sand (2:1) and maintained in a greenhouse at 28 ºC with sterile nutrition solution (Norris and T'mannetje, 1964) during the growth period.

Heat shock treatment: Fourteen-days-old plants, grown at 28 ºC, were submitted to 2-4 h of heat shock treatment at 40 ºC in a growth chamber. Leaves were collected after 10 and 30 min and 1 and 2 h of stress. Following the end of the heat shock treatment (2 h), the plants were maintained at 28 ºC for a recovery period of up to 4 h and leaves were again collected after every hour. The leaves were immediately frozen in liquid nitrogen and stored at –70 ºC until total RNA extraction.

RNA extraction and analysis: Total RNA was isolated from each sample of frozen tissue (0.5 g) by the method adapted from Ragueh et al. (1989). After grinding in liquid nitrogen, the material was resuspended in 0.6 mL of an extraction buffer (200 mM Tris, pH 7.0, 200 mM EDTA, 1 % SDS), submitted twice to extraction with 1 volume of phenol:chloform:isoamylalcohol (25:24:1) and finally to extraction with 0.6 mL phenol. The aqueous phase was separated by centrifugation (10 min, 12,000 rpm). After extraction with phenol, 0.75 mL of LiCl (4 $mol.L^{-1}$) was added to the aqueous phase and the mixture was held overnight at 4 ºC. The precipitated RNA was pelleted by centrifugation (20 min, 12,000 rpm). The pellet was washed three times with ethanol (75 %) and resuspended in 50 µL of sterile distilled water. The RNA concentration was determined by measuring absorbance at 260 nm.

For Northern analysis, 20 µg of total RNA of each sample was submitted to electrophoresis in formaldehyde-containing agarose gels (1,5 %) as described by Sambrook et al. (1989). After electrophoresis, RNA was blotted onto nitrocellulose membrane and hybridized with W HSP 16.9 cDNA clone labeled with [^{32}P]CTP as a probe. The hybridization signal intensities were quantified from the X-ray film using a LKB Laser densitometer. The values in

Table 1. Main characteristics of the common bean varieties used.

Common bean variety	Seed		Phaseolin protein type	Growth habit	Growth Temperature (ºC)
	color	size			
IPA 7 [a]	Cream	Media	-	Indeterminate, semi-climbing, III	38
Negro Argel [b]	Black	Small	-	Indeterminate, semi-climbing, III	-
Jamapa [b]	Black	Small	S	Indeterminate upright, erect II	24
ChimaltenangroNegro [b]	Black	Small	S	Indeterminate, climbing IV	18
Flor de Mayo [b]	Pink	Media	S	Indeterminate, semi-climbing, III	22
Cacahuate [b]	Cream	Media	T	Indeterminate, semi-climbing, III	18
Cranberry [b]	Cream	Media	C, H	Indeterminate, semi climber III	22
Canario [b]	Yellow	Media	T	Determinate prostrate, or upright, I	22

[a] Communicated by IPA-Empresa Pernambucana de Pesquisa Agropecuária; [b] Singh (1989)

"counts" (1/8 $V.s^{-1}$) for each band were used to follow the mRNA accumulation kinetics. In order to compare the expression pattern of the different varieties, values were corrected by the intensity of ethidium bromide staining of the rRNA in the agarose gels, measured as described.

Protein extraction and analysis by western blot: After the heat shock treatment, total protein was extracted from each sample of frozen tissue (0.5 g) according to the method described by Lin et al. (1984). SDS-PAGE was performed using a V16 model cube from GibcoBRL and an electrophoresis power supply model Power Pac 300 (Bio-Rad). Total protein suspensions on aliquots were mixed with an equal volume of loading buffer (0,1 $mol.L^{-1}$ Tris-HCl pH 6.8, 4.1 M β-mercaptoethanol, 11.4 % SDS, 0.1 % bromophenol blue) and loaded on 12.5 or 15 % polyacrylamide gels. Electrophoresis was carried out at 35 mA for 2 h and at 180 V for a further 4 h period. Proteins were stained with Coomassie Brilliant Blue-R250.

The proteins separated by SDS-PAGE were electrophoretically transferred to a 0.45 µm nitrocellulose membrane by means of a mini Protein II cell transfer apparatus for western blotting detection. The transference was performed in a transfer buffer (0.025 $mmol.L^{-1}$ Tris-HCl, 125$mmol.L^{-1}$ glycine, 20 % methanol, pH 8.3) during 1 h at 100 V. The membrane was incubated with 50 mL of blocking buffer (100 $mmol.L^{-1}$ phosphate buffer pH 7.0, 5 % nonfat dry milk). The specific anti-rice 16.9 sHSP (Jinn et al., 1993) was added to a final concentration of 1:1500 and incubation took place during 2 h. The membrane was then washed 3 times with 40 mL of TBS and incubated for 1 h with anti-rabbit IgG coupled with alkaline phosphatase diluted to 1:500 in blocking buffer. The detection was performed by BCIP and NBT (Bio-Rad).

RESULTS

Kinetics of accumulation of HSP mRNAs in different common bean varieties: The patterns of SHSP mRNA accumulation in response to heat stress were examined for 8 common bean varieties using Northern blot experiments. Cowpea was also used as a reference since this plant grows quite well under high temperature regimes. As no bean or cowpea sHSP cDNA is available to be used as an homologous probe, cDNA from a wheat heat shock protein with 16.9 kDa (McElwain and Spiker 1989), which in a previous experiment showed strong positive hybridization with common bean mRNA, was used to detect sHSP transcripts in both leguminous plants. The results indicate the existence of at least three different patterns of induction and accumulation of sHSP mRNAs when plants were submitted to 40 ºC during 2 h (figures 1 and 2). The HSP mRNA induction was observed as soon as the plants were transferred to a 40 ºC chamber, while maximum accumulation was observed after 1 to 2 h from the beginning of the heat shock. After 2 h, the plants were transferred back to a green house where temperature was kept between 28 and 30 ºC. Plants were harvested for an extra period of 4 h, during the recovery period when it was observed significant levels of mRNA hybridizing with W HSP 16.9 probe.

sHSP mRNAs were expressed within 10 min of heat shock at 40 ºC for all varieties but not in Negro Argel (figure 1 A-I). After 30 min of heat shock, a continuous increase in the mRNA levels hybridizing with W HSP 16.9 was observed, the highest levels being reached for most of the varieties after 1 h of the heat treatment. Varieties Canario and Negro Argel showed the maximum level of transcript after 2 h of the heat shock treatment (figures 1E and 1H). For cowpea plants, the maximum level of transcript was reached after 1 hour, which was maintained until the end of the heat shock treatment. Furthermore, for cowpea the transcript level accumulated was considerable higher than that observed for common bean varieties. On the other hand, for varieties Flor de Mayo, Cranberry and IPA 7 that showed the maximum level of transcript after 1 h of heat shock, a decrease in the sHSP mRNA level was observed after this period, although the heat shock was still being applied. After removing the plants from the 40 ºC chamber, they were allowed to recover at 28 ºC during 4 h. The sHSP mRNA from common bean varieties reduced significantly after 2 h on the recovery temperature. This reduction was less accentuated for cowpea and Canario varieties.

The autoradiography of the Northerns blot membranes were scanned with a laser densitometer and the values corresponding to the intensities of W HSP 16.9 mRNA hybridization band were recorded. These values were plotted against time for each variety studied (figure 2). The different varieties could be arranged on three groups according to the pattern of steady state of sHSP mRNA levels reached in response to heat shock. Group 1 is

represented by cowpea and the IPA 7 variety, which displayed the highest level of transcript accumulation (figure 2A). Group 2 (figure 2B) is represented by Cranberry, Canario and Cacahuate, which express intermediate transcript levels. Group 3 encompasses most of the common bean varieties and is characterized by a small amount of transcript formation that occurs during the first hour of heat treatment, disappearing quickly after that (figure 2C).

Accumulation of HSP in cowpea and in common bean**.** The patterns of sHSP accumulation in response to heat stress were determined for 2 common bean varieties and cowpea by western blot using the anti-rice sHSP 16.9 (Jinn et al., 1993). The antibody raised from 16.9 rice HSP cross-reacts with sHSP of a large number of plant species including wheat, soybean, mung bean and others (Jinn et al., 1993). The sHSP mRNAs accumulated in the plant leaf tissues used in this experiment were also analyzed by Northern blot using the W HSP 16.9 cDNA (McElwain and Spiker, 1989). The results confirmed that for both cowpea and IPA 7 bean variety the patterns of sHSP mRNA accumulation are very similar, showing a maximum mRNA level after 2 h and a fast decrease after 4 h from the onset of the treatment. In contrast, Negro Argel bean variety presented a smaller but very stable accumulation level of the sHSP mRNA during the observed period (figure 3B). The western blotting analysis showed that the accumulation levels of sHSPs are higher in IPA 7 and cowpea than in Negro Argel, although, the protein amount in bean IPA 7 variety decreased faster than in cowpea. Besides that, cowpea presented, in addition to the 16.9 kDa protein, a second band at 22 kDa revealed by the anti-rice sHSP. For Negro Argel variety, there was a strict correlation between the accumulation pattern of sHSP mRNAs and the protein concentration when plants were submitted to the 40 ºC shock treatment (figures 3A and 3B).

The western blot analysis confirmed that cowpea and the IPA 7 bean variety, displayed the highest level of accumulation of sHSP while Negro Argel was characterized by a weak expression of this proteins (figure 3A).

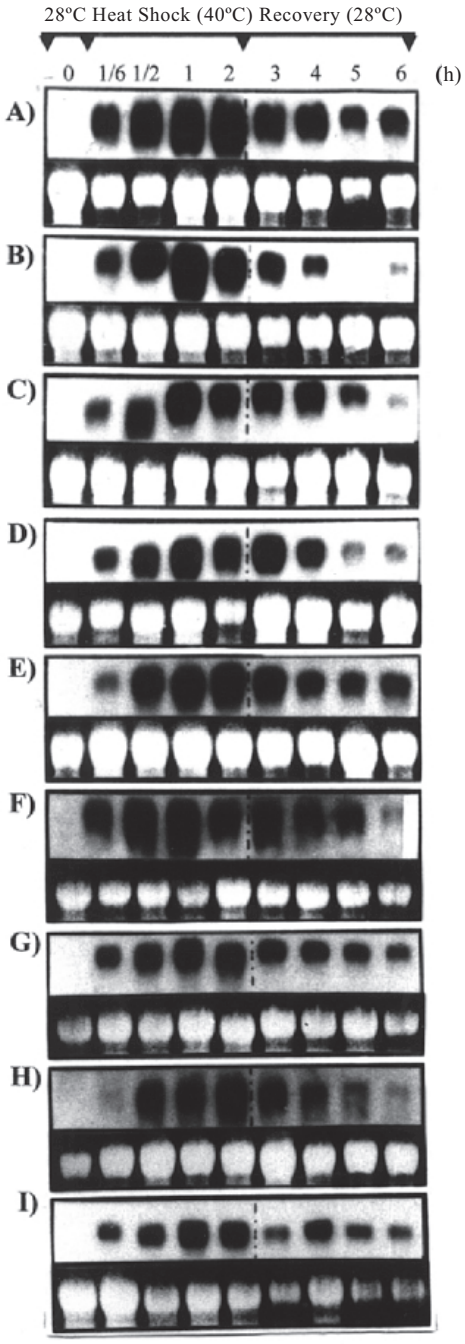

Figure 1. Accumulation of sHSPs during heat shock and recovery periods. Northern blot of total RNA from leaves of cowpea and different common bean varieties. The plants were submitted to heat shock at 40 ºC up to 2 h and then transferred to 28 ºC up to 4 h. The W HSP 16.9 cDNA was used as probe. A) Cowpea, B) IPA 7, C) Cranberry, D) Cocahuate, E) Canario, F) Flor de Mayo, G) Chimaltenango Negro, H) Negro Argel, I) Jamapa. Lower panel: ethidium bromide staining of ribosomal RNAs to verify equivalency of mRNA loading.

DISCUSSION

The accumulation of sHSP mRNAs in cowpea and eight common bean varieties was evaluated by Northern blot using a heterologous probe W HSP 16.9 isolated from wheat (McElwain and Spiker, 1989). The percentage of

amino acid similarity among class I sHSPs, isolated from different species of plants, was shown to be very high reaching values over 90 % (Jinn et al., 1993). Thus, it was not astonishing that heat shock stress was capable of inducing mRNA, which hybridizes with the heterologous probe, even under high stringency conditions, in all bean varieties used in this work. The analysis of sHSP mRNA accumulation showed considerable differences among the distinct bean varieties in response to heat shock. The transcripts were detected as soon as ten minutes after the beginning of the heat shock for most of the varieties, except for Negro Argel and Canario.

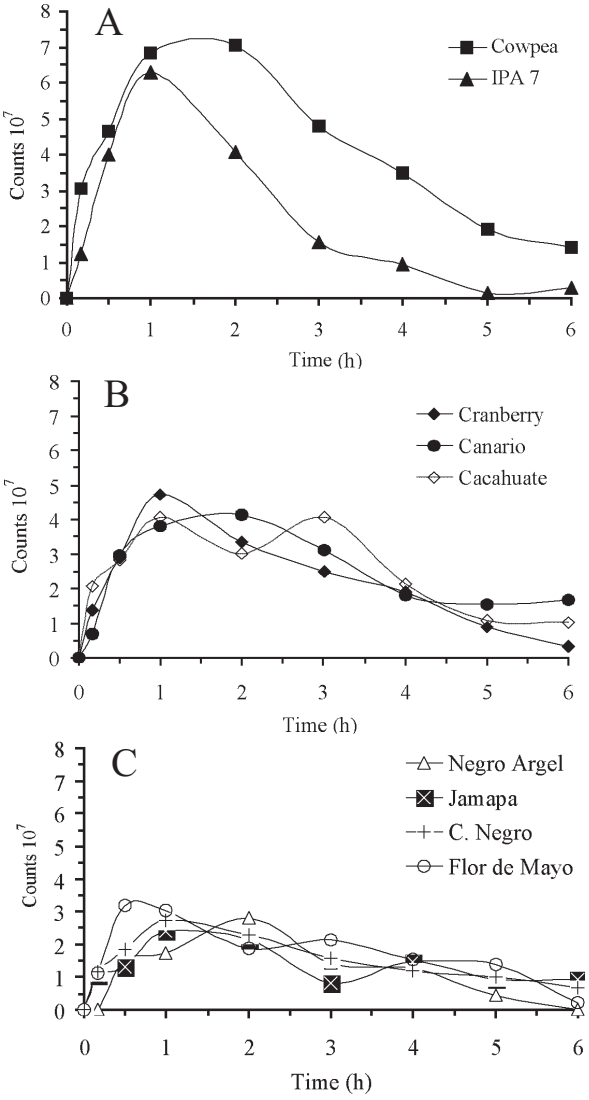

Figure 2. Kinetics of accumulation of sHSP mRNA during heat shock and further recovery period. Each value corresponds to the hybridization signal intensity from the Northern normalized by the values obtained from the rRNA both measured by densitometry.

The induction of sHSP in response to heat shock was previously observed in several species, such as wheat (Weng and Nguyen, 1992), pea (DeRocher et al., 1991; Lenne and Douce, 1994) and corn (Nieto-Sotelo et al., 1989). Lenne and Douce (1994) studied the sHPS gene expression in pea mitochondria and observed that the maximum accumulation of mRNA occurred within the first hour of treatment at 40 ºC. Similar results were also obtained by Nieto-Sotelo et al. (1989) when examining the levels of HSP 26 mRNA during heat shock treatment. Considering that the presence of sHSPs can prevent denaturation caused by high temperature (Jinn et al., 1993; Yeh et al., 1995) the fast accumulation of them could play an important role on the protection of the metabolic apparatus of the cell. Therefore, reduction of deleterious effects promoted by high temperature could be a key factor for organism adaptation under these conditions. Jinn et al. (1997) showed evidence that sHSPs are essential for acquisition of thermotolerance in two-day-old soybean seedlings. Recently, Malik et al. (1997a) have demonstrated that both carrot cell lines and tomato plants transformed with HSP 17.7, a small heat shock protein from carrot, exhibit increased thermotolerance. The same group also showed a substantially reduction on thermotolerance with cell lines that were expressing the antisense to the HSP 17.7 gene in relation to control cells. Analysis of the antisense transfected cells revealed that HSPs protein synthesis was reduced and the effect was even more intense with the low molecular weight class (Malik et al., 1997b).

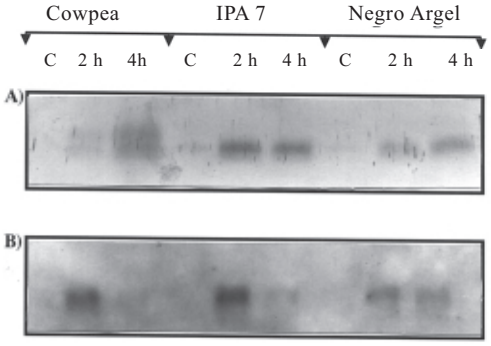

Figure 3. sHSP accumulation during heat shock. The plants were submitted to heat treatment at 40 ºC for 2 and 4 h. The control plants were kept at 28 ºC. (A) Western blot of total leaf protein from cowpea and two varieties of bean (IPA7 and Negro Argel). The anti rice sHSP 16.9 was used as specific antibody. (B) Northern blot of total RNA from the same plants used in "A". The W HSP 16.9 cDNA was used as probe.

Some bean varieties studied here showed a reduction in the sHSP transcript level after the first hour of the heat shock despite of this treatment being still applied. Studies on pea also showed that, after a maximum HSP mRNA level has been reached, degradation of the transcripts became more intense than synthesis resulting in a decrease of the detected mRNA. Nevertheless this decreasing is not necessarily followed by a reduction in the protein level (Lenne et al., 1995). Differences in the kinetics of synthesis and degradation of HSPs and its mRNAs have been reported (Kimpel et al., 1990; Green, 1993). The results presented here suggest that sHSP mRNA half-life may be dependent on the bean genotype. The variety IPA 7 has the half-life of sHSP mRNAs limited to some hours. On the other hand, half-life for pea sHSP proteins can be as long as 52 h (DeRocher et al., 1991) and for soybean seedling more than 3 d (Jinn et al., 1997). The observed decrease on the HSP mRNA levels does not necessarily correlate with the HSP concentration (Apuya and Zimmerman, 1992). Nevertheless, our results from the western blotting analysis suggested that the sHSPs levels on the three genotypes studied; cowpea, bean IPA 7 and Negro Argel, are coordinated with the kinetics of mRNA accumulation, indicating that the major control point of sHSP expression corresponds to the transcriptional process. Despite the use of cDNA probe and antibody from different sources, wheat and rice, respectively, the antibody raised from 16.9 kDa rice HSP cross-reacted with wheat sHSP (Jinn et al., 1993). Thus, we could expect that the bean proteins reacting with rice sHSP antibody belong to the same class revealed by the wheat sHSP cDNA.

The pattern of the mRNA accumulation during the recovery period shows substantial differences among the distinct bean genotypes. Cowpea and three bean varieties (Canario, Cacahuate and Jamapa) maintained a high quantity of transcripts after 4 h of recovering at 28 ºC. However, for the other varieties studied the transcript level decreased drastically as soon as the high temperature stress was removed. Among them, IPA 7 has a unique pattern, presenting a higher accumulation at the beginning of the temperature treatment, but the mRNA rapidly decrease after the end of this treatment. Varieties Flor de Mayo, Chimaltenango Negro, Jamapa and Negro Argel show a very low level of mRNA accumulation during the heat stress. Gurley and Key (1991) reported that mRNA stability is the main factor involved in the maintenance of HSP mRNA levels after the end of the stress period. Therefore, the results obtained during and after the stress period may suggest that mRNA stability can be modulated according to the bean variety analyzed.

Although the mechanisms by which HSPs provide thermotolerance are still unknown, the differences observed on the transcript and protein accumulation levels suggest that different varieties present distinct response to heat stress. The studies reported by Weng and Nguyen (1992) using different varieties of wheat showed that W HSP 16.9 and other HSPs probes were capable of hybridizing more intensively with thermotolerant varieties. In this work, we have used cowpea as a control since this leguminous plant can be cultivated under high temperature conditions. Although none of the bean varieties studied displayed a behavior similar to cowpea concerning sHSP mRNA accumulation, IPA 7 was considered to have the closest pattern. This variety was recently recommended for the Northeast region of Brazil where high temperature regimes prevail.

The parameters studied allowed the identification of three main groups of bean varieties regarding the level of mRNA induced by heat stress. One group is represented by variety IPA 7, which induces the highest level of HSP mRNAs during the stress period. Corroborating with this result, the IPA 7 variety is tolerant to high temperatures and recommended to be cultivated at 38 °C (communicated by IPA-Empresa Pernambucana de Pesquisa Agropecuária, September, 1990). Varieties Cacahuate, Canaria, Cranberry and Flor de Mayo are representatives of a group with intermediate level of HSP mRNA induction, while Chimaltenango Negro, Jamapa and Negro Argel produce the lowest level of transcript during heat stress. All varieties from groups 2 and 3 present optimal growing temperature between 18 and 24 °C (table 1). Evans (1973) reported that a difference on thermotolerance among the varieties might be observed accordingly to the original domestication area. The varieties originated from the Meso-america region, showing predominantly growth habit types II and III, are more tolerant to high temperature than varieties originated from the Andean, characterized by growth habits I and IV well adapted to high altitudes and cold weather. The same pattern is described to lima bean (*Phaseolus lunatus*) cultivars where the Mesoamericam gene pool have greater adaptability to heat stress than cultivars from the Andean gene pool (Mackie, 1943, cited by Keeler et al., 2000). Among the varieties used in this study, three are from Mesoamerica region and two are from the Andea. In this work, varieties of Mesoamerica origin

and habit type III, presented a higher induction of HSP mRNAs indicating a relationship between transcript production and thermotolerance.

Yield under heat stress may be affected by various aspects of the heat response, such as, the ability to detect temperature as stressful, the ability to response shifts quickly, the extent of the response, and the tissue specificity of the response (Keeler et al., 2000). The importance of the HSPs in the heat stress tolerance is still not completely understood but somehow they are involved on tolerance to several environmental stresses. In the literature, the response of bean plants to heat stress is still poorly represented and furthers studies are needed since the characterization of bean heat shock related genes and their expression might be an important contribution for the assisted selection of bean varieties adapted to tropical conditions.

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